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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/890,319	07/26/2001	Olivier Dhellin	265/130	2012

34313 7590 09/09/2003

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EXAMINER	
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ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 09/09/2003

7

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/890,319	DHELLIN ET AL.	
	Examiner	Art Unit	
	Karen A Canella	1642	

-- The MAILING DATE of this communication appears on th cover sheet with the c rrespond nce address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1-14,17-19,21,22 and 25-27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-14,17-19,21,22 and 25-27* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *attachment A and B*

DETAILED ACTION

1. Claims 16, 20, 23 and 24 have been canceled. Claims 26 and 27 have been added. Claims 1, 2, 4-15, 17-19, 21, 22 and 25 have been amended. Claims 1-14, 17-19, 21, 22 and 25-27 are pending and under consideration.

2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action

3. Applicants communication on the FAX dated April 16, 2003 has been noted. Accordingly, a copy of the PTO 948 form is enclosed with this action. Please note that Applicant is required to submit the drawing corrections within the period for reply set in the attached Office Action summary.

4. The objection to claims 4, 8, 9, 10, 13, 14, 15, 16, 20, 21, 22 and under 37 CFR 1.75(c) as being improper multiple dependent claims is withdrawn in light of applicants amendments.

5. The rejection of claims 5 and 7 under 35 U.S.C. 102(b) as being anticipated by the abstract of Michaelson et al (Monographs in Neural Sciences, 1980, Vol. 7, pp. 19-29) as evidenced by Alberts et al (Molecular Biology of the Cell, 1989, pp. 1077-1079) is withdrawn in light of applicants amendments

6. The rejection of claims 5 and 7 under 35 U.S.C. 102(b) as being anticipated by Dubinsky et al (American Journal of Physiology, 1986, Vol. 251, pp. C713-C720) as evidenced by Langridge-Smith et al (Biochimica et Biophysica Acta, 1984, Vol. 777, pp. 84-92) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) is withdrawn in light of applicants amendments.

7. The rejection of claims 5 and 7 under 35 U.S.C. 102(b) as being anticipated Von der Decken (European Journal of Biochemistry, 1968, Vol. 4, pp. 87-94) as evidenced by Gordon

(Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and Smith et al (Oxford Dictionary of Biochemistry and Molecular Biology, 1997, page 419) and the abstract of Nishino et al (Archives of Biochemistry and Biophysics, 2000, Vol. 374, pp. 293-298) and the abstract of Tanaka et al (Journal of Biological Chemistry, 1987, Vol. 262, pp. 1374-1381) and the abstract of Seeger (Z. Krebsforsch, 1950, Vol. 57, pp. 113-120) is withdrawn in light of applicants amendments

8. The rejection of claims 1, 2, 5, 7 and 23 under 35 U.S.C. 102(b) as being anticipated by Vaandrager et al (Biochimica et Biophysica Acta, 1988, Vol. 939, pp. 305-314) as evidenced by the Sigma Catalog, (1997, page 1801) is withdrawn in light of applicants amendments

9. The rejection of claims 1, 5, 7 and 23 under 35 U.S.C. 102(b) as being anticipated by Denning et al (Journal of Protozoology, 1989, vol. 36, pp. 334-340) is withdrawn in light of applicants amendments

10. The rejection of claims 1, 3, 5-7, 11, 12 and 23 under 35 U.S.C. 102(b) as being anticipated by Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) as evidenced by the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted July 26, 2001) is withdrawn in light of applicants amendments.

11. The rejection of claims 1-3, 5-7, 11, 12, 17-19 and 23 35 U.S.C. 103(a) as being unpatentable over Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) as evidenced by the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization,

1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted July 26, 2001), in view of Zitvogel et al (WO 99/03499, reference AC of the I.D.S. filed July 26, 2001) and Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) and Pharmacia Biotechnology (Ion Exchange Chromatography, Principles and Methods, 1991, 3rd edition, page 34) is withdrawn in light of applicants amendments.

12. The rejection of claims 1-3, 5-7, 11, 12, 17-19, 23 and 24 under 35 U.S.C. 103(a) as being unpatentable over Feldman et al and the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD of the I.D.S. submitted July 26, 2001) and Zitvogel et al (WO 99/03499, reference AC of the I.D.S. filed July 26, 2001) and Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) and Pharmacia Biotechnology (Ion Exchange Chromatography, Principles and Methods, 1991, 3rd edition, page 34), as applied to claims 1-3, 5-7, 11, 12, 17-19 and 23 above, and further in view of Zitvogel et al (Nature Medicine, 1998, Vol. 4, pp. 594-600) and Amigorena (Hematology and Cell Therapy, 1997, Vol. 39, pp. 87-89) and Ogle et al (U.S. 6,165,785) is withdrawn in light of applicants amendments.

13. The rejection of claims 1, 3, 5-7, 11, 12, 23 and 24 under 35 U.S.C. 103(a) as being unpatentable over Feldman et al (PNAS, 1987, vol. 84, pp. 6775-6779) and the Sigma Catalog (1997, page 1815) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and the abstract of Reuveny et al (Developments in Biological Standardization, 1980, vol. 46, pp. 137-145) and Abbas et al (Cellular and Molecular Immunology, (text), 1991, page 17) and Raposo et al (Journal of Experimental Medicine, 1996, Vol. 183, pp. 1161-1172, reference AD

of the I.D.S. filed July 26, 2001) in view of Olge et al (U.S. 6,165,785) is withdrawn in light of applicants amendments .

14. The objection to claims 4, 8-10, 13-16, 20-22 and 25 as improper multiply-dependent claims is withdrawn in light of applicants amendments. Accordingly, the claims are no longer withdrawn from consideration.

New Grounds of Rejection

15. Claim 2 is objected to because of the following informalities: the typographical error of "witha". Appropriate correction is required.

16. Claim 4 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 3. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). The scope of claim 4 is identical to the scope of claim 3.

17. Claim 9 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "low" in claim 9 is a relative term which renders the claim indefinite. The term "low" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. The specification does not provide a limiting definition for determining the metes and bounds of "low speed" centrifugation. The specification states on page 11, lines 24-29 that preferred embodiments of low speed centrifugation are "below 1000 g" and between 100 and 700 G. However, these preferred embodiments do not constitute an absolute limitation for "low speed centrifugation".

18. Claim 25 is rejected under 35 U.S.C. 102(b) as being anticipated by Raposo et al (Journal of Experimental Medicine, 1996, Vol..183, pp. 1161-1172, cited in the previous Office Action)

or Zitvogel et al (Nature Medicine, 1998, Vol. 4, pp. 594-600, cited in the previous Office Action). Claim 25 is drawn to a composition comprising membrane vesicles prepared using the process according to claim 1. Claim 1 is drawn to a process of preparing membrane vesicles from a biological sample, wherein said biological sample comprises membrane vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens, said method comprising at least one anion-exchange chromatography treatment. Thus, claim 25 is a product by process claim.

Raposo et al disclose a composition of exosomes prepared from a B lymphocyte cell line (EBV-B) which were incubated in the presence of HSP-protein from M. leprae (page 1163, first column, lines 1-9 under the heading "Antigen Presentation Assays"). Raposo et al disclose that the exosomes were prepared by means of differential centrifugation (page 1163, first column lines 8-9 and page 1162, second column, lines 9-13 under the heading "Biotinylation"), rather than anion exchange chromatography. However, it appear that the exosomes prepared by differential centrifugation would have the same properties as exosomes prepared by anion exchange chromatography.

Zitvogel et al teach the preparation of exosomes from dendritic cells which were pulsed with tumor peptides (pages 595 to 596, bridging sentence). The pulsed dendritic cells would therefore be sensitized to antigens wherein said antigens are the tumor peptides. Zitvogel et al teach that dendritic cell exosomes were prepared by differential centrifugation (page 65, lines 10-15). However, it appears that the dendritic cell exosomes prepared by differential centrifugation would have the same properties as exosomes prepared by anion exchange chromatography.

Section 2113 of the MPEP states:

> Product-by-process claims are not limited to the manipulations of the recited steps, only the structure implied by the steps. "[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985)

Thus, it is the applicants burden to provide evidence establishing an unobvious difference between the claimed product and the prior art products. In re Marosi, 710 F.2d 798, 802, 218 USPQ 289, 292 (Fed. Cir.1983).

19. Claims 26 and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zitvogel et al (WO 99/03499) as evidenced by the attached translation (PTO-2-5049) in view of Dubinsky et al (American Journal of Physiology, 1986, Vol. 251, pp. C713-C720, cited in the previous Office Action) and Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103, cited in the previous Office Action). Claim 26 is drawn to a process of preparing membrane vesicles from a biological sample comprising the culture of a population of membrane vesicle-producing tumor cells under conditions enabling the release of vesicles, a membrane vesicle enrichment step and either an anion exchange chromatography step or a gel permeation chromatography step. Claim 27 embodies the method of claim 26 wherein the tumor cells are human tumor cells.

Zitvogel et al teach that texosomes are membrane vesicles obtained from vesicle producing tumor cells (page 6, line 8 to page 7, line 12 of the translated document). Zitvogel et al teach that said texosomes are characterized by a lipid bilayer surrounding a cytosolic fraction. Zitvogel et al teach a process of preparing texosomes wherein the isolation stage may be carried out using chromatography (page 18, lines 20-22 of the translation). Zitvogel et al teach that the use of chromatography enables the production of higher quantities of the vesicles for pharmacological uses (page 35, lines 11-16). Zitvogel et al teach human tumor cells which produces texosomes (page 55, lines 2-5), thus fulfilling the specific embodiment of claim 27 with regard to the human origin of the cells. Zitvogel et al specifically teach gel permeation chromatography for the preparation of dexosomes (page 35, lines 4-7), however, Zitvogel do not specifically teach the use of gel permeation chromatography in the process of preparing the texosomes.

Dubinsky et al disclose a process of preparing membrane vesicles comprising passing said enriched vesicle preparation through a Sephadex G-50 column (abstract, lines 8-10 and page 85, second column, lines 12-18). Gordon (page 102, lines 5-7 and 13-18) discloses that the use of Sephadex to separate substrates based on size is described as "gel permeation" chromatography.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to purify the texosomes by gel permeation chromatography. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Dubinski et al on the purification of membrane vesicles by passing though a Sephadex column, and the teachings of Zitvogel et al directing the isolation of texosomes by means of chromatography to enable the production of higher quantities of vesicles for pharmacological use, and the further eachings of Zitvogel on the use of gel permeation chromatography in the process of preparing dexosomes. .

20. Claims 5, 7 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Raposo et al (Journal of Experimental Medicine, 1996, Vol..183, pp. 1161-1172) in view of Von der Decken (European Journal of Biochemistry, 1968, Vol. 4, pp. 87-94, cited in the previous Office action) as evidenced by Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and Smith et al (Oxford Dictionary of Biochemistry and Molecular Biology, 1997, page 419) and the abstract of Nishino et al (Archives of Biochemistry and Biophysics, 2000, Vol. 374, pp. 293-298) and the abstract of Tanaka et al (Journal of Biological Chemistry, 1987, Vol. 262, pp. 1374-1381) and the abstract of Seeger (Z. Krebsforsch, 1950, Vol. 57, pp. 113-120).

Claim 5 is drawn to a process of preparing membrane vesicles from a biological sample wherein said process comprises at least the culture of a population of membrane vesicles producing antigen presenting cells under conditions enabling the release of vesicles, wherein said antigen presenting cells have been sensitized to one or more antigens, treatment of the ample to prepare a sample enriched in membrane vesicles, and an anion exchange or gel permeation chromatography step. Claim 7 embodies the process of claim 5 wherein the enrichment step comprises a clarification stage. Claim 9 embodies the process of claim 5 wherein the enrichment step comprises a low speed centrifugation step or a filtration. It is noted that claim 9 is rejected under 112, second paragraph because the metes and bound of "low speed centrifugation" cannot be determined from the specification.

Raposo et al teach a process of preparing membrane vesicles from a B lymphocyte cell line (EBV-B) which were incubated in the presence of HSP-protein from M. leprae (page 1163,

first column, lines 1-9 under the heading "Antigen Presentation Assays"). Raposo et al disclose that the exosomes were prepared by means of differential centrifugation followed by flotation on a sucrose gradient (page 1163, first column lines 8-9 and page 1162, second column, lines 9-13 under the heading "Biotinylation"). The instant specification (page 11, lines 12-14) states that "the enrichment step comprises (i) the elimination of cells and/or cell debris(clarification)", therefore differential centrifugation fulfills the specific embodiment of clarification. Raposo et al do not teach the preparation of vesicles from B cells by means of gel permeation chromatography.

Von der Decken teaches a process for preparing an enriched sample of microsomes comprising centrifugation of homogenized liver to obtain a supernatant, followed by layering of said supernatant onto a sucrose layer and further centrifugation to obtain a clear supernatant and a microsomal pellet (page 88, first column, first paragraph under the heading "Preparation of Microsomes, Ribosomes and Cell Sap"). Thus the centrifugation of the homogenized liver suspension fulfills the specific embodiment of claim 7 drawn to a clarification stage. Von der Decken discloses that the enriched sample of microsomes was further purified by passing through a column of Sephadex G-25 (abstract, lines 5-6 and page 88, under the heading "Molecular Sieving through Sephadex G-25"). Von der Decken teaches that the passing of the microsomes through Sephadex G-25 further removed "components and factors" from the microsomal sample (abstract, lines 5-6).

The specification defines membrane vesicles as vesicles composed of a lipid bilayer containing a cytosolic fraction (page 1, lines 6-8). Smith et al define a microsome as a vesicle formed from disrupted membranes of the endoplasmic reticulum and the plasma membrane. The abstract of Nishino et al states (last sentence) that the microsomal membrane is composed of a lipid bilayer. The abstract of Seeger defined cytoplasmic granules as comprising microsomes, and the abstract of Tanaka et al describes a cytoplasmic surface within a microsomal sphere. Thus, microsomes are vesicular structures which possess a lipid bilayer and contain a cytosolic fraction and as consistent with the definition in the specification for membrane vesicles..

Gordon (page 102, lines 5-7 and 13-18) discloses that the use of Sephadex to separate substrates based on size is described as "gel permeation" chromatography.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to further purify the membrane vesicles taught by Raposo et al by gel permeation chromatography by passing said vesicles through Sephadex G25. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Von der Decken et al on the improvement in purity of microsomes obtained from a process comprising the additional step of passing the microsomes through Sepahdex-G25 after centrifugation through sucrose.

21. Claims 5, 7, 9 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Raposo et al (Journal of Experimental Medicine, 1996, Vol..183, pp. 1161-1172, cited in the previous Office Action) in view of Von der Decken (European Journal of Biochemistry, 1968, Vol. 4, pp. 87-94) as evidenced by Gordon (Electrophoresis and Chromatography, In: The Origins of Modern Biochemistry, Annals of the New York Academy of Sciences, 1979, Vol. 325, pp. 95-103) and Smith et al (Oxford Dictionary of Biochemistry and Molecular Biology, 1997, page 419) and the abstract of Nishino et al (Archives of Biochemistry and Biophysics, 2000, Vol. 374, pp. 293-298) and the abstract of Tanaka et al (Journal of Biological Chemistry, 1987, Vol. 262, pp. 1374-1381) and the abstract of Seeger (Z. Krebsforsch, 1950, Vol. 57, pp. 113-120) as applied to claims 5, 7 and 9 above, and further in view of Lenk et al (WO 89/00846).

The specific embodiments of claims 5, 7 and 9 are set forth above along with the teachings of Raposo et al, Von der Decken, Gordon, Smith et al, the abstract of Tanaka et al and the abstract of Seeger et al which render obvious the embodiments of said claims.

Claim 10 embodies the process of claim 5 wherein the enrichment comprises at least a tangential ultra filtration step.

Neither Raposo et al, Von der Decken, Gordon, Smith et al, the abstract of Tanaka et al nor the abstract of Seeger et al teach a process for the preparation of membrane vesicles comprising a tangential ultrafiltration step.

Lenk et al teach a process for the preparation of plurilamellar vesicles based on size separation by a tangential flow filtration unit (abstract). Lenk et al teach that this method is particularly useful for liposomes as it avoids the physical damaging of liposomes while

allowing for the concentration of said liposomes within the retentate thus allowing for the recovery of a liposomal product at a desired concentration (page 10, line 36 to page 11, line 8)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to include a separation step in the method rendered obvious by the combination of Raposo et al, Von der Decken, Gordon, Smith et al, the abstract of Tanaka et al and the abstract of Seeger et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Lenk et al on the use of tangential ultrafiltration to allow for the retention of a liposomal preparation while smaller molecules pass through the membrane, thereby allowing for the concentration of the membrane vesicles without physical damage to said vesicles.

22. Claims 1-7, 9, 13-15, 17-19, 21, 22 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zitvogel et al (WO 99/03499, reference AC of the I.D.S. filed July 26, 2001, cited in the previous Office Action) in view of Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610, cited in the previous Office Action) and Vaandrager et al (Biochimica et Biophysica Acta, 1988, Vol. 939, pp. 305-314) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182, cited in the previous Office Action) as evidenced by the Sigma Catalog, (1997, pages 1801-1803). The specific embodiments of claims 5, 7 and 9 are set forth above. Claim 1 is drawn to a process of preparing membrane vesicles for a biological sample wherein said biological sample comprises membrane vesicles produced by antigen presenting cells that have been sensitized to one or more selected antigens, said method comprising at least one anion exchange treatment. Claim 2 is drawn to the process of claim 1 wherein anion exchange chromatography is performed on a support functionalized with a quaternary amine. Claims 3 and 4 embody the process of claim 1, wherein the biological sample is a biological fluid, a culture supernatant a cell lysate or a pre-purified solution. Claim 13 embodies the process of claim 1 wherein the membrane vesicles have a diameter between approximately 60 and 90 nm. Claim 14 embodies the process of claim 1 wherein the antigen presenting cells comprise dendritic cells, B lymphocytes, macrophage or mastocytes. Claim 25 is drawn to the composition comprising the membrane vesicles prepared using the process of claim 1.

Claim 6 is drawn to a process of preparing membrane vesicles from a biological sample comprising the culture of a population of dendritic cells under conditions enabling the release of membrane vesicles, a membrane vesicle enrichment step and either anion exchange chromatography or gel permeation chromatography. claim 15 is drawn to the process of claim 6 wherein the membrane vesicles are produced by human dendritic cells.

Claim 17 is drawn to a process of preparing membrane vesicles comprising obtaining a population of cells comprising immature dendritic cells and culturing said cells under conditions enabling the release of membrane vesicles and purifying the membrane vesicles using a process comprising at least one anion exchange chromatography treatment.

Claim 18 is drawn to a process of preparing membrane vesicles comprising obtaining a population of immature dendritic cells, culturing said cells under conditions enabling the release of membrane vesicles, treating the supernatant to produce a biological sample enriched in membrane vesicles and purifying said vesicles by means of either an anion exchange or gel permeation chromatography step. Claim 19 embodies the process of claim 18 wherein the dendritic cells are obtained from a biological sample from a subject. Claim 21 embodies the process of claim 17 wherein the dendritic cells are sensitized to antigen prior to the membrane vesicle production step. Claim 22 embodies the process of claim 17 wherein the dendritic cells are culture under conditions stimulating membrane vesicle production in step b.

Zitvogel et al (1999) teach dexosomes as membrane vesicles derived from dendritic cells (abstract and title). Zitvogel teach that said dexosomes are preferentially obtained from immature dendritic cells (page 26, lines 19-23) which can be obtained from the culture supernatants of dendritic cells (page 22, lines 9-15), and wherein said dexosomes have a diameter ranging generally from 60-90 nm (page 23, lines 14-16). Zitvogel et al teach that a preferred embodiment is preparation of dexosomes from dendritic cells that have been sensitized to an antigen or a group of antigens (page 28, lines 7-11). Zitvogel et al teach obtaining dendritic cells from monocyte precursors taken from a subject or the direct isolation of dendritic cells (page 25, lines 4-8 and lines 21-23). Zitvogel et al suggest ion exchange chromatography, gel permeation chromatography and ultrafiltration (nanofiltration) for the purification of dexosomes (page 27, lines 15-20 of the original document). Zitvogel et al teach that due to the nature of the

dexosome lipid membrane, ion exchange chromatography is especially of interest (page 35, lines 8-10 of the translation).

Zitvogel et al do not specifically teach anion exchange as the specific type of ion exchange chromatography.

Thiery et al teach conditions for producing dexosomes from dendritic cells (page 603, under the heading "Exosomes production during DC maturation"). Thiery et al teach human monocyte-derived dendritic cells (page 603, second column, last sentence), thus fulfilling the specific embodiments of claims 15 and 19 drawn to human dendritic cells and dendritic cells obtained from a subject. Thiery et al teach a clarification step using protein G-Sepharose (page 601, under the heading "Metabolic Labeling of Cells and Exosomes, lines 13-17), thus fulfilling the specific embodiment of claim 7, drawn to a clarification step.

Thiery et al do not teach anion exchange chromatography, gel permeation or ultrafiltration to isolate or purify the dendritic cell exosomes. However, Thiery et al teach that exosomal membranes have exposed phosphatidylserine residues (page 607, last paragraph, lines 7-9).

Chen et al teach that polyunsaturated acid-containing molecular species of phosphatidylserine can be isolated from bovine brain extract by means of anion exchange chromatography without loss of the polyunsaturated moiety (abstract). Chen et al teach that Q-Sepharose was the anion exchange resin (page 179, first column under the heading "Chemicals"). Pharmacia Biotechnology (page 34, second paragraph) identifies Q Sepharose as a strong anion exchanger.

Vaandrager et al disclose a process of preparing membrane vesicles comprising a 1X-8 anion-exchange column (abstract, lines 2-5 and page 306, second column, third paragraph). The anion exchange column used by Vaandrager et al is a quaternary amine as evidenced by pages 1802-1803 of the Sigma Catalog which indicate that the 1X-8 anion exchanger is trimethylbenzylammonium (page 1802, second column, under the heading "Dowex 1 Strongly Basic Anion Exchangers and page 1803, first column, "1X-8-50") and that the anion exchanger is on polystyrene (page 1801, "Group 4: anion exchangers on polystyrene"), thus fulfilling the specific embodiments of claim 2 drawn to a support functionalized with a quaternary amine.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to prepare the dendritic cell exosomes by a method comprising anion exchange chromatography on a support functionalized with a quaternary amine. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the suggestion of Zitvogel et al that dendritic cell derived exosomes be purified by ion-exchange chromatography due to the nature of the dendritic cell exosomal membrane; the teaching of Thiery et al on the presence of exposed phosphatidylserine in the membrane of exosomes; and the teachings of Chen et al on a process for purifying molecular species comprising phosphatidylserine by the use of anion exchange chromatography on Q Sepharose, a quaternary amine anion exchanger and the teachings of Vaandrager et al on the preparation of membrane vesicles by a method comprising the 1X-8 anion exchanger.

23. Claims 1-4, 5, 6-9, 11, 13-15, 17-19, 21, 22 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zitvogel et al (WO 99/03499, Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610), Vaandrager et al (Biochimica et Biophysica Acta, 1988, Vol. 939, pp. 305-314) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) as evidenced by the Sigma Catalog, (1997, pages 1801-1803) as applied to claims 1-4, 5, 6, 7, 9, 13-15, 17-19, 21, 22 and 25 above, and further in view of Zitvogel et al (Nature Medicine, 1998, Vol. 4, pp. 594-600) and Amigorena (Hematology and Cell Therapy, 1997, Vol. 39, pp. 87-89) and Ogle et al (U.S. 6,165,785). The specific embodiments of claims 1-4, 5, 6, 7, 9, 13-15, 17-19 and the teachings of the prior art reference that render obvious said claims are set forth above. Claims 8 embodies the method of claim 5 wherein the enrichment step comprises an affinity chromatography step. Claim 11 is drawn to a process of preparing membrane vesicles comprising the culturing of a population of antigen presenting cells under conditions enabling the release of membrane vesicles, the treatment of the culture supernatant with at least one affinity chromatography step to produce a biological sample enriched with membrane vesicles and an anion exchange or a gel permeation chromatography treatment. Neither of Zitvogel et al (WO 99/03499, Thiery et al, Vaandrager et al nor Chen et al teach an affinity chromatography step as applied to a process of preparing membrane vesicles.

Thiery et al teach that exosomes produced by dendritic cells exposed to tumor-derived antigenic peptides induce potent immune responses, such as cytotoxic T-lymphocytes, leading to the regression of established tumors in mice (page 600, first column, second full paragraph). Thiery et al teach that dendritic cell-derived exosomes have both MHC class II and MHC class I molecules. Thiery et al teach that batches of exosomes derived from culture of murine dendritic cells were contaminated by bovine exosomes present in the fetal calf serum used for the culture medium (page 600, second column, second paragraph under the heading "Exosome Purification"). Thiery et al teach that the actual presence of murine derived dendritic cell exosomes was demonstrated by immunoprecipitation. The legend for figure 7 states that exosomes were immunoprecipitated with antibodies specific to MHC II.

Zitvogel et al (1998) teach that administration of dendritic cell derived exosomes loaded with tumor peptides results in tumor growth delay or tumor eradication when administered to tumor-bearing mice. Zitvogel et al teach that these exosomes contained syngeneic MHC molecules (page 595, second column, last line to page 596, first column line 1). Zitvogel et al teach that the administration of exosomes bearing allogenic MHC molecules did not induce significant anti-tumor responses (page 597, first column, lines 13-16).

Amigorena teaches that dendritic cell exosomes bear CD63 and CD82 markers absent from the dendritic cell surface (page 87, second column, lines 1-3).

Olge et al teach that enriched populations can be obtained from mixed cell suspensions by positive selection (column 21, lines 16-17) and that the technology for capturing specific cells on affinity materials is well known in the art. Ogle et al further teach that procedures for separation of cells may include affinity chromatography.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to purify membrane vesicles produced by dendritic ells by affinity chromatography using an antibody directed toward the CD63 or CD82 molecule of the syngenic dendritic exosome. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Thiery et al on the contamination of dendritic cell exosomes with bovine exosomes, the teaching of Zitvogel et al on the usefulness of syngenic, versus allogenic, dendritic exosomes in the treatment of tumors, the teachings of Amigorena on the presence of CD63 and CD82 exclusively on the surface of dendritic cell

exosomes, the teachings of Olge et al on the general utility of separations using affinity materials. One of skill in the art would be motivated to use affinity chromatography in order to remove contaminating bovine exosomes because the contaminated preparations would evoke an anti-bovine response when administered in vivo and because the contaminating bovine exosomes would not present tumor-derived peptides in the context of syngeneic MHC molecules which are taught by Zitvogel et al (1998) to be necessary to evoke an anti-tumor response.

24. Claims 1-15, 17-19, 21, 22 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zitvogel et al (WO 99/03499, Thiery et al (Journal of Cell Biology, 1999, vol. 147, pp. 599-610), Vaandrager et al (Biochimica et Biophysica Acta, 1988, Vol. 939, pp. 305-314) and Chen et al (Journal of Chromatography, 1995, vol. 666, pp. 178-182) and Zitvogel et al (Nature Medicine, 1998, Vol. 4, pp. 594-600) and Amigorena (Hematology and Cell Therapy, 1997, Vol. 39, pp. 87-89) and Ogle et al (U.S. 6,165,785) as applied to claims 1-4, 5, 6-9, 11, 13-15, 17-19, 21, 22 and 25 above, and further in view of Lenk et al. The specific embodiments of claims 1-4, 5, 6-9, 11, 13-15, 17-19, 21, 22 and 25 and the teachings of the prior art references that render obvious the instant claims are set forth above. Claim 10 is drawn to the process of claims 5 or 6 wherein the enrichment step comprises a tangential ultrafiltration step. claim 12 is drawn to the process of claim 11 further comprising a sterilization filtration in step d of the treated preparation.

Neither Zitvogel, Thiery, Vaandrager et al, Chen et al, Amigorena or Olge et al teach a method of preparing membrane vesicles comprising a sterilization filtration.

Lenk et al teach tangential flow filtration comprising a first filter of about 0.2 microns which excludes particles larger than the desired cutoff, for the preparation of sterile liposomes (page 13, lines 33-35 and page 23, lines 7-8).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to sterilize the membrane vesicles from dendritic cells by means of tangential flow filtration through a 0.2 micron membrane. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Lenk et al on the sterilization of compositions comprising liposomes.

25. Applicant argues that the combination of references in the rejection under 35 U.S.C. 103(a) as set forth in the previous Office action do not render obvious the instant invention , because it was unexpected that the membrane vesicles would retain integrity of the exterior proteins after chromatography treatments. This has been considered but not found persuasive. It is recognized in the art that membrane vesicles can be purified by gel permeation and ion exchange chromatography. It was also inherent in the references which teach the preparation of the membrane vesicles, that the product obtained by the chromatographic procedure was functional and the membrane vesicles were not damaged. Further, Zitvogel et al teaches that the membrane vesicles obtained from dendritic cells be purified by chromatography, especially ion-exchange chromatography. Zitvogel also suggests that the membrane vesicles obtained from the tumor cells be purified by means of chromatography. given the success of the prior art in isolating purified membrane vesicles by chromatography, and the suggestion from Zitvogel that it be carried out for the preparation of membrane vesicles derived from dendritic cells and tumor cells, one of skill in the art would have a reasonable expectation of success with the methods rendered obvious by the combination of the prior art references. Thus Zitvogel provides motivation to explicitly combine the references. Further, Von der Decken teaches that chromatography of microsomes on Sephadex columns further purified the microsomes after differential gradient centrifugation. Thus, one of skill in the art would be motivated to use a chromatographic purification to obtain a preparation of membrane vesicles of higher purity. Applicant argues that the combination of references do not anticipate each and every limitation of the claimed methods (page 21, last three lines). However, applicant does not point out the specific embodiment that was not taught by the combination of references.

26. All other rejections and objections as set forth in Paper No. 3 are withdrawn.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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9/4/03